

### Short Communication

## Analysis of DNA and Hemoglobin Adducts and Sister Chromatid Exchanges in a Human Population Occupationally Exposed to Propylene Oxide: A Pilot Study

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### Abstract

Propylene oxide (PO), a simple alkylating agent used in the chemical industry, is weakly genotoxic and induces nasal cavity tumors in rodents on inhalation at high air concentrations. DNA adducts, hemoglobin adducts, and sister chromatid exchanges (SCE) were analyzed as biomarkers of exposure in a group of eight PO-exposed workers and eight nonexposed subjects. 1-2-Hydroxypropyladenine (1-HP-adenine) in DNA of WBCs was analyzed using a hypersensitive <sup>32</sup>P-postlabeling assay. HP-valine in hemoglobin was measured using gas chromatography/tandem mass spectrometry. Air measurements indicated PO levels in the range of 1–7 ppm. All three biomarkers showed significantly increased levels in the exposed workers. 1-HP-adenine was recorded in seven of the exposed workers (mean 0.66 mol/10<sup>9</sup> mol nucleotides) but was not detected in any of the control subjects. HP-valine was found in all subjects (means of 2.7 and 0.006 pmol/mg globin in exposed workers and controls, respectively). The average frequencies of SCE were 3.7/cell in exposed workers and 2.0/cell in controls, respectively. DNA and hemoglobin adducts were correlated ( $r = 0.887$ ), as well as DNA adducts and SCE ( $r = 0.792$ ) and hemoglobin adducts and SCE ( $r = 0.762$ ). The present study is the first demonstrating PO-DNA adducts in human individuals. It is also the first study indicating cytogenetic effects in humans from PO exposure, although confounding effects from other sources cannot be excluded.

### Introduction

PO<sup>2</sup> is a common industrial chemical mainly used for production of propylene glycols, polyether polyols, and propylene glycol ethers. The Swedish exposure limit for PO is 2 ppm, and in the United States, the Occupational Safety and Health Administration has set a limit of 20 ppm, and the American Conference of Governmental Industrial Hygienists limit is set at 2 ppm. PO is weakly mutagenic and clastogenic and induces nasal cavity tumors in rodents on inhalation at high doses (1, 2).

PO is a direct alkylating agent introducing HP groups on reactions with nucleophilic sites in DNA and proteins (2–4), and such adducts could therefore be used to monitor PO exposures (5). The major adduct formed in DNA after *in vitro* reaction of PO is 7-HP-guanine, followed by the adducts 3-HP-, 1-HP-adenine, and 3-HP-cytosine (3, 6). These adducts are chemically unstable: 7-HP-guanine and 3-HP-adenine will depurinate, forming apurinic sites, and 1-HP-adenine and 3-HP-cytosine will spontaneously convert to N<sup>6</sup>-HP-adenine and 3-HP-uracil, respectively (3, 6). PO adducts have been analyzed recently in rats exposed to PO by inhalation. 7-HP-guanine was analyzed by gas chromatography/mass spectrometry and by <sup>32</sup>P-postlabeling (7, 8); the minor adducts have only been detected using the latter assay (6). Significant repair of either 7-HP-guanine or 1-HP-adenine was not seen in those animal studies. The level of 1-HP-adenine corresponded to ~2% of 7-HP-guanine, but the recovery in the postlabeling assay exceeded that of 7-HP-guanine (50 versus 12%). In addition, 1-HP-adenine was chemically more stable ( $t_{1/2} = 9.2$  days for rearrangement to an N<sup>6</sup> adduct compared with  $t_{1/2} = 5$  days for depurination of 7-HP-guanine; Ref. 6). Therefore, it was suggested that this minor adduct could be an alternative for monitoring PO exposures (6).

Hemoglobin is the protein most commonly used for monitoring exposures to chemical carcinogens. PO adducts in hemoglobin are chemically stable, with the same life span as the erythrocytes themselves (~4 months in humans). Hemoglobin adducts have been measured in humans after occupational exposure to PO (5, 9). DNA adducts from this compound have not been determined in human populations this far. Chromosomal aberrations, micronuclei, and unscheduled DNA synthesis have been analyzed in a human population exposed to PO (10–12). An effect on unscheduled DNA synthesis was observed, but the lack of a control population made it impossible to establish an effect of exposure for the cytogenetic data. The aim of the present study was, therefore, to measure both DNA and hemoglobin adducts and SCE in a small group of workers occupationally exposed to PO.

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<sup>2</sup> The abbreviations used are: PO, propylene oxide; HP, 2-hydroxypropyl; SCE, sister chromatid exchange; HPLC, high-performance liquid chromatography.

## Materials and Methods

**Sample Collection.** Venous blood (5–10 ml) was collected from eight PO-exposed workers at a PO-producing plant and from an equal number of control subjects at an institute of occupational health in the Liaoning province, People's Republic of China. The mean age was 29 (range 26–34) for the exposed group and 31 (range 26–33) for the control subjects. The individuals in the exposed group had worked 7–10 years in the factory, with  $\geq 2$  years with the present duties. There was an equal distribution of sexes, and there were two smokers in each group. PO air samples were collected on activated charcoal tubes for 20–60 min and analyzed by gas chromatography. Historical exposure measurements suggest that the levels were  $\sim \leq 10$  ppm. Stationary air measurements recorded 1 day before blood sample collection revealed the highest levels in the packing area (3.7–6.9 ppm). In some common rooms and in the area of polymerization, the levels were 0.9–1.7 ppm. The workers spend 1–1.5 h/day in a packing area, transferring PO from larger reservoirs through pipes to containers of  $\sim 100$  liters and the rest of the 8-h working day in the common rooms not performing work duties related to PO or other exposures.

Blood samples were collected in two heparinized glass tubes. One tube was frozen directly and used for later isolation of WBC DNA (7). The other tube was used for setting up the cell culture for cytogenetic analysis and isolation of globin.

**Adduct Analyses.** 1-HP-adenine was analyzed by  $^{32}\text{P}$ -postlabeling as described (6). Postlabeled samples were mixed with the synthetically prepared standard 1-HP-5'-dAMP and separated by HPLC. The standard was detected by UV, and the peak was collected and pooled from four runs, each based on 10  $\mu\text{g}$  of DNA from the same person. The pooled sample was heated in 0.1 M NaOH (80°C, 30 min) and reanalyzed by HPLC with online UV and radioisotope detection. This treatment quantitatively converted 1-HP-5'-dAMP to  $N^6$ -HP-5'-dAMP. A DNA sample modified *in vitro* with  $^{14}\text{C}$ -labeled PO was used as an external standard to determine the recovery of 1-HP-adenine in the postlabeling assay (6). When analyzing this DNA, a recovery through the whole postlabeling procedure of 50% was obtained. The detection limit (after correcting for recovery) was 0.1 adduct/ $10^9$  nucleotides. All human samples were coded before analysis, and each sample was analyzed at least twice.

HP-valine was measured using the *N*-alkyl Edman method as described previously (13). All samples were coded before analysis. Hemoglobin was only obtained from six of the eight control individuals. Each sample was analyzed once. Previous studies have demonstrated a good reproducibility of the method with a SD of  $< 5\%$  of the mean.

**Cytogenetic Analysis.** Each culture was set up by adding 0.5 ml of whole blood into 5 ml of culture medium containing F10 medium (Life Technologies, Inc., Breda, the Netherlands), 20% FCS (heat inactivated; Life Technologies, Inc.), heparin (32 units/ml; Sigma Chemical Co., St. Louis, MO), L-glutamine (1.65 mM; Life Technologies, Inc.), antibiotics (100 units/ml penicillin and 0.1 mg/ml streptomycin), and phytohemagglutinin (0.3 mg/ml; Murex, Dartford, United Kingdom). 5-Bromo-2'-deoxyuridine (BrdUrd, 10  $\mu\text{M}$ ; Sigma Chemical Co.) was added, and the cultures were incubated for 72 h at 37°C in a 95% humidified incubator with 5%  $\text{CO}_2$ . Air-dried metaphase preparations were made in a routine way.

Sister chromatid differentiation using fluorescence plus Giemsa staining was not successful, indicating that the level of incorporated BrdUrd was low. Therefore, an anti-BrdUrd antibody was used for detecting SCEs. The slides were denatured for 1 min in 0.07 M NaOH and dehydrated in an ethanol series

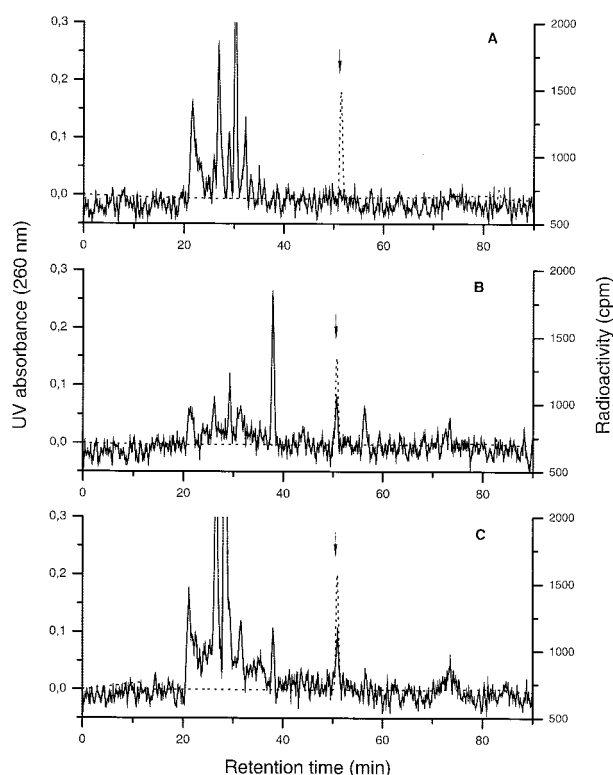


Fig. 1. HPLC separation of  $^{32}\text{P}$ -postlabeled DNA from WBCs of a control person (A) and PO-exposed workers (B and C). The position of  $N^6$ -HP-5'-dAMP is indicated with an arrow. —, radioactivity; ---, UV. The HPLC system was first run isocratically with 100% of 0.5 M ammonium formate containing 20 mM phosphoric acid (pH 4.6) for 5 min, followed by a linear gradient to 15% methanol over 40 min, isocratically with 15% methanol for 20 min, and finally, a linear gradient to 100% of methanol over 15 min.

(70, 90, and 100%). Visualization of chromatids incorporated with BrdUrd was accomplished with anti-BrdUrd mouse antibody (Boehringer, Mannheim, Germany) and Alexa fluor 488 antimouse antibody (Molecular Probes, Eugene, OR) as described (14). Samples were coded before scoring.

## Results

After separation of the alkaline-treated samples by HPLC, a radioactivity peak showing cochromatography with the UV peak of  $N^6$ -HP-adenine was detected in seven of eight exposed workers but not in any of the controls. Some representative HPLC radiograms are shown in Fig. 1. The adduct levels ranged from undetectable to 1 mol (mean  $0.66 \pm 0.34$  mol) per  $10^9$  mol normal nucleotides. For statistical calculations, an adduct level of  $0.05/10^9$  (50% of the detection limit) was used for samples with undetectable levels. The adduct levels in samples from PO workers were significantly higher than those of the controls (Mann-Whitney two sample test,  $P = 0.0012$ ). The adduct HP-valine was found in all human samples; the levels ranged from 0.005 to 0.008 pmol/mg globin among the controls and from 0.13 to 4.92 (mean  $2.69 \pm 1.52$ ) pmol/mg globin among the exposed workers. The effect of exposure was highly significant ( $P = 0.0018$ ). The mean SCE frequency was  $3.7 \pm 2.11$  for the exposed workers and  $2.0 \pm 0.52$  for the control group. The difference was significant ( $P = 0.011$ ). Because the two smokers in the control group were the indi-

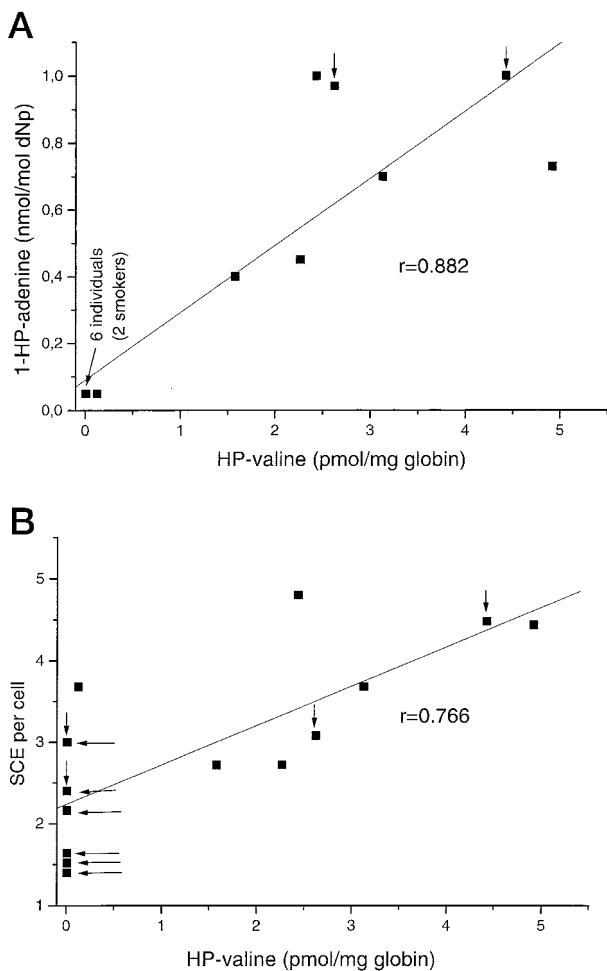


Fig. 2. Correlation between levels of hemoglobin and DNA adducts (A) and between frequencies of SCEs and levels of hemoglobin adducts (B). Data for unexposed individuals are indicated by a horizontal arrow and for smokers by a vertical arrow.

individuals with the highest SCE frequency, the difference between exposed and controls was increased if excluding smokers ( $P = 0.0038$ ). The correlation between levels of 1-HP-adenine and HP-valine was strong ( $r = 0.887$ ) and highly significant ( $P < 0.0001$ ; Fig. 2A). There was also a relatively strong correlation between levels of 1-HP-adenine and SCE frequencies ( $r = 0.792$ ;  $P = 0.00026$ ) and between HP-valine and SCEs ( $r = 0.766$ ;  $P = 0.0014$ ; Fig. 2B), respectively. The two control persons for which HP-valine was not analyzed (and, therefore, not included in the correlation analysis) had both 1.96 SCE/cell, *i.e.*, very close to the mean of the unexposed group. Excluding smokers, the correlation between SCE and HP-adenine was 0.851 ( $P = 0.00044$ ), and the correlation between SCE and HP-valine was 0.757 ( $P = 0.011$ ). Excluding the control subjects, the correlation between 1-HP-adenine and HP-valine was 0.713 ( $P = 0.047$ ).

## Discussion

We had earlier developed and applied a very sensitive method for detection of adducts of PO at 1-adenine (6). The same method has also been used for analysis of 1-adenine adducts in

a human population exposed to butadiene (15). The main reasons for the high sensitivity were the high recovery of the adduct in the  $^{32}\text{P}$ -postlabeling assay ( $\sim 50\%$  for PO) and the procedure used for analysis. Analysis of 1-HP-adenine was difficult because interfering unknown radioactivity peaks eluted very close to the standard. However, by collecting the 1-HP-adenine peak and converting 1-HP-adenine to  $N^6$ -HP-adenine, sensitivity and specificity was increased. With this procedure, it was possible to analyze a greater amount of DNA (40 versus 10  $\mu\text{g}$ ), and one adduct per  $10^{10}$  nucleotides could be reliably detected. Furthermore, by performing the conversion to an  $N^6$ -adenine adduct, the evidence for correct adduct identification was strengthened. This rearrangement also occurs spontaneously (3, 6), but we found, contrary to Solomon *et al.* (3), that the rate at physiological conditions was rather slow in PO-modified DNA (half-life of 9.2 days; Ref. 6). An additional support for a slow rate is the finding that after 4 weeks of chronic exposure of rats to PO, only some 20% of 1-HP-adenine had rearranged to  $N^6$ -HP-adenine (6).

The two-step procedure used for analysis together with the finding that all except one of the PO-exposed workers and none of the control subjects had detectable adduct levels support the contention that the adduct analyzed was formed as a consequence of PO exposure and that it was indeed 1-HP-adenine. The exposed worker who showed no detectable HP-adenine had also a considerably lower level of HP-valine than the rest of the exposed workers (see Fig. 2).

HP-valine could be detected in all samples, but the levels in control subjects were  $\sim 600$  times lower than in PO workers. Assuming the same ratio for 1-HP-adenine, one would expect the level of this adduct in controls to be on the order of 100 times below detection limit of the postlabeling assay. Still, background levels of HP-valine were above the detection limit of the assay, showing the superior sensitivity of hemoglobin adduct measurements compared with the most sensitive assay for DNA adduct analysis. HP-valine in PO-exposed workers has been analyzed by Boogaard *et al.* (9). The adduct levels per unit dose were close to those reported here. We found an average adduct level of 2.7 pmol/mg globin in the eight PO plant workers, and the average air concentration of PO was estimated to 2.3 ppm during working hours. Boogaard *et al.* (9) found an adduct increment of 2.55 pmol/gram globin/ppmh, corresponding to an accumulated adduct level of 2.1 pmol/mg globin for an occupational exposure to 2.3 ppm. The low background level of HP-valine shows that contributions from nonoccupational sources are minor. The origin of HP-valine in the control subjects is unknown, but propene is a likely candidate chemical. This simple alkene, which is metabolically converted to PO (16), is present at ppb levels in air contaminated with tobacco smoke or automobile exhausts (17). There were only two smokers in the unexposed group, and their smoking habits are unknown. The levels of HP-valine of the smokers were similar to those of the nonsmokers, indicating that tobacco smoking is not the major contributing factor to the found background levels.

Induction of SCE after PO exposure has thus far only been studied in cells *in vitro* and experimental animals (18–21). PO induced SCE in cellular systems and in the mouse but not in the cynomolgus monkey (18). The SCE frequencies found in the control group of this study were lower than normally observed (22). Possibly, the low baseline frequency, as well as the low induced frequencies of SCEs, were because of the low incorporation of BrdUrd, which could be detected only by immunological techniques. It has been shown that frequencies of SCEs are directly correlated to the level of incorporated BrdUrd

(14). Excluding smokers strengthened the effect of PO exposure and only marginally changed the correlation between SCE and Hb or DNA adducts.

Poly(propylene glycol) monobutyl ether was the only other chemical reported to be handled by the workers. This compound has to our knowledge not been tested, but judged from testing of related substances, it is not expected to be genotoxic. Therefore, it is likely that the observed increase in SCE occurred as a result of PO exposure, but confounding effects from genotoxic impurities of the polymer or other unknown sources cannot be excluded.

Bearing in mind that DNA and hemoglobin adducts and SCE integrate exposure for different time intervals, the good correlation between all three end points was somewhat unexpected. One possible explanation is that the exposure levels had not varied much during the last months. Although the present study was of small scale, the presence of a DNA adduct not seen previously in humans was demonstrated, as was the usefulness of the studied end points for monitoring PO exposures at levels at or below current exposure limits.

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### References

- IARC. Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans, Vol. 60, pp. 181–213. Lyon, France: IARC, 1994.
- Ríos-Blanco, M. N., Plná, K., Faller, T., Kessler, W., Håkansson, K., Kreuzer, P. E., Ranasinghe, A., Filser, J. G., Segerbäck, D., and Swenberg, J. A. Propylene oxide: its relationship to mutagenesis, carcinogenesis and molecular dose. *Mutat. Res.*, 380: 179–197, 1997.
- Solomon, J. J., Mukai, F., Fedyk, J., and Segal, A. Reactions of propylene oxide with 2'-deoxynucleosides and *in vitro* with calf thymus DNA. *Chem. Biol. Interact.*, 67: 275–294, 1988.
- Svensson, K., Olofsson, K., and Osterman-Golkar, S. Alkylation of DNA and hemoglobin in the mouse following exposure to propene and propylene oxide. *Chem. Biol. Interact.*, 78: 55–66, 1991.
- Osterman-Golkar, S., Bailey, E., Farmer, P. B., Gorf, S. M., and Lamb, J. H. Monitoring exposure to propylene oxide through the determination of hemoglobin alkylation. *Scand. J. Work Environ. Health*, 10: 99–102, 1984.
- Plná, K., Nilsson, R., Koskinen, M., and Segerbäck, D. <sup>32</sup>P-postlabelling of propylene oxide 1- and N<sup>6</sup>-substituted adenine and 3-substituted cytosine/uracil: formation and persistence *in vitro* and *in vivo*. *Carcinogenesis (Lond.)*, 20: 2025–2032, 1999.
- Segerbäck, D., Plná, K., Faller, T., Kreuzer, P. E., Håkansson, K., Filser, J. G., and Nilsson, R. Tissue distribution of DNA adducts in male Fischer rats exposed to 500 ppm of propylene oxide: quantitative analysis of 7-(2-hydroxypropyl)-guanine by <sup>32</sup>P-postlabelling. *Chem. Biol. Interact.*, 115: 229–246, 1998.
- Ríos-Blanco, M. N., Faller, T. H., Nakamura, J., Kessler, W., Kreuzer, P. E., Ranasinghe, A., Filser, J. G., and Swenberg, J. A. Quantitation of DNA and hemoglobin adducts and apurinic/aprimidinic sites in tissues of F344 rats exposed to propylene oxide by inhalation. *Carcinogenesis (Lond.)*, 21: 2011–2018, 2000.
- Boogaard, P. J., Rocchi, P. S. J., and van Sittert, N. J. Biomonitoring of exposure to ethylene oxide and propylene oxide by determination of hemoglobin adducts: correlations between airborne exposure and adduct levels. *Int. Arch. Occup. Environ. Health*, 72: 142–150, 1999.
- Pero, R. W., Bryngelsson, T., Widegren, B., Högstedt, B., and Welinder, H. A reduced capacity for unscheduled DNA synthesis in lymphocytes from individuals exposed to propylene oxide and ethylene oxide. *Mutat. Res.*, 104: 193–200, 1982.
- Pero, R. W., Osterman-Golkar, S., and Högstedt, B. Unscheduled DNA synthesis correlated to alkylation of hemoglobin in individuals occupationally exposed to propylene oxide. *Cell Biol. Toxicol.*, 1: 309–314, 1985.
- Högstedt, B., Bergmark, E., Törnqvist, M., and Osterman-Golkar, S. Chromosomal aberrations and micronuclei in relation to alkylation of hemoglobin in workers exposed to ethylene oxide and propylene oxide. *Hereditas*, 113: 133–138, 1990.
- Osterman-Golkar, S., Licea Perez, H. L., Csanady, G. A., Kessler, W., and Filser, J. G. Methods for biological monitoring of propylene oxide exposure in Fischer 344 rats. *Toxicology*, 134: 1–8, 1999.
- Natarajan, A. T., Rotteveel, A. H. M., van Pieterse, J., and Schliermann, M. G. Influence of incorporated 5-bromodeoxyuridine on the frequencies of spontaneous and induced sister-chromatid exchanges, detected by immunological methods. *Mutat. Res.*, 163: 51–55, 1986.
- Zhao, C., Vodicka, P., Sram, R. J., and Hemminki, K. Human DNA adducts of 1,3-butadiene, an important environmental carcinogen. *Carcinogenesis (Lond.)*, 21: 107–111, 2000.
- Svensson, K., and Osterman-Golkar, S. Kinetics of metabolism of propene and covalent binding to macromolecules in the mouse. *Toxicol. Appl. Pharmacol.*, 73: 363–372, 1984.
- IARC. Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans, Vol. 60, pp. 161–180. Lyon, France: IARC, 1994b.
- Lynch, D. W., Lewis, T. R., Moorman, W. J., Burg, J. R., Gulati, D. K., Kaur, P., and Sabharwal, P. S. Sister-chromatid exchanges in chromosome aberrations in lymphocytes from monkeys exposed to ethylene oxide and propylene oxide by inhalation. *Toxicol. Appl. Pharmacol.*, 76: 85–95, 1984b.
- Tucker, J. D., Xu, J., Stewart, P. C., Baciu, T., and Ong, T. Detection of sister chromatid exchanges induced by volatile genotoxicants. *Teratog. Carcinog. Mutagen*, 6: 15–21, 1986.
- Agurell, E., Cederberg, H., Ehrenberg, L., Lindahl-Kiessling, K., Rannug, U., and Törnqvist, M. Genotoxic effects of ethylene oxide and propylene oxide: a comparative study. *Mutat. Res.*, 250: 229–237, 1991.
- Farooqi, Z., Törnqvist, M., Ehrenberg, L., and Natarajan, A. T. Genotoxic effects of ethylene oxide and propylene oxide in mouse bone marrow cells. *Mutat. Res.*, 288: 223–228, 1993.
- Tates, A. D., Grummt, T., Törnqvist, M., Farmer, P. B., van Dam, F. J., van Mossel, H., Schoemaker, H. M., Osterman-Golkar, S., Uebel, Ch., Tang, Y. S., Zwiderman, A. H., Natarajan, A. T., and Ehrenberg, L. Biological and chemical monitoring of occupational exposure to ethylene oxide. *Mutat. Res.*, 250: 483–497, 1991.